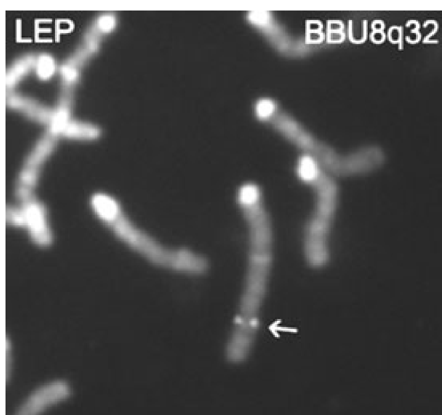

Figure 1 Fluorescence *in situ* hybridization mapping of the bubaline leptin gene. Specific signals were detected on chromosome BBU8q32 (arrow). The prometaphase detail of river buffalo chromosomes shows simultaneous disclosure of RBPI-banding and FITC-signals.

normally responsible for intracellular trafficing of the synthesized protein and changes in the primary structure could therefore have an influence on the intracellular transport efficiency. In our study all buffaloes had a glutamine at this site.

Another difference was found in the promoter region. Whereas in cattle there is a putative Sp1 binding site 3 basepairs downstream the TATA box, this site is not present in buffaloes because of a G to A exchange. Mutation at another SpI site in the promoter region of the murine leptin (-98 bp of the first base of exon 1) was shown to result in a 2.5-fold reduced promoter activity.[8] The physiological consequence of the amino acid difference between cattle and buffalo is currently unknown.

Acknowledgements: We gratefully acknowledge financial support from CAPES, CNPq, BASA, DAAD and the Erxleben Research & Innovation Council (ERIC-BR1959-2000-1). Alexandra Siebels is thanked for expert technical assistance.

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Acc. No. AY495586; AY495587; AF387813; AF387814

doi:10.1111/j.1365-2052.2004.01188.x

Development of type I markers in channel catfish through intron sequencing

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Accepted for publication 9 August 2004

Source and description: Type I markers are useful for comparative gene mapping, but they have not been widely available in catfish.[1] Here we took the approach of sequencing the intron sequences of genes with the assumption that the introns are more prone to mutations and may have higher chances of containing microsatellites. Primers were designed to amplify introns of 50 genes, of which 42 produced amplicons that were successfully sequenced (Table 1).

PCR conditions and genotyping: In initial reactions to amplify the intron fragments for sequencing, PCR reactions were not labelled. A 50 μl PCR reaction was prepared containing primers at 2 ng/μl each, dNTPs at 0.1 mM each, 200 ng template DNA, 1X PCR reaction buffer (Invitrogen, Carlsbad, CA, USA), 1.5 mM $MgCl_2$ and 2 units of *Faststart Taq* DNA polymerase (Roche, Mannheim, Germany). Cycling conditions were denaturation at 95 °C for 1 min, annealing at a temperature specific to each pair of primers (Table 1) for 30 s, and extension at 72 °C for 1 min, for 40 cycles with a final extension at 72 °C for 10 min using an MJ Research PTC100 thermocycler. For analysis of polymorphism in outbred channel catfish and for linkage map genotyping, PCR products were fluorescently labelled and sized on an ABI PRISM 3100 Genetic Analyzer.[2]

Sequencing analysis of PCR products: Seven of the 42 PCR amplicons were cloned into pCR2.1-TOPO (Invitrogen) and then sequenced using M13 universal and reverse sequencing primers with a LI-COR automated sequencer. The remaining 35 PCR amplicons were sequenced directly using both of the original PCR primers on an ABI Prism 3100 Genetic Analyzer. All sequences have been deposited in GenBank with accession numbers listed in Table 1.

Polymorphisms and linkage mapping: To assess the utility of eight microsatellite-containing clones, heterozygosities were determined by genotyping 33–37 individuals using outbred catfish from commercial populations. The number of alleles and observed heterozygosities are summarized in Table 2. Of the eight gene-associated microsatellites, *IpSTS00006* failed to produce scorable PCR products and was eliminated from further analysis. Genotypes from the seven remaining gene-associated microsatellite loci were obtained from full-sib reference families 1 and 2, and linkage was determined using CRI-MAP.[3] Six of the seven genes mapped to specific linkage groups[3]

Table 1 PCR primers, PCR conditions, sequences, and identification of microsatellites from the amplicons of 42 gene-derived sequences.

STS no.	Accession no.	Gene identity	PCR primers	Annealing temp °C	Introns	Size of intron (bp)	Microsatellite
IpSTS00001	BV078112	*Translation elongation factor 2*	tgtggatgttaccaagggagtgc ccacaaacataggagttcccatgacc	61	1, 2	93, 80	(tatt)7
IpSTS00002	BV078113	*Isocitrate dehydrogenase 3*	gaggaagctggacctgtttg cttggtggcgtagtcaaagg	55	2	573	(ac)18
IpSTS00003	BV078114	*TA2 t-complex polypeptide 1*	agaagttgttcaggatagggtgtgtg ggttttgaccattgtgggctcg	58	2, 3	143, 308	(tg)8
IpSTS00004	BV078115	*SEC61, alpha subunit*	gctaaacagcttaaggagcagcag gctcccaaaaaatcggccatgac	61	3	761	(tttg)7
IpSTS00005	BV078116	*cytochrome P450 aromatase*	cgcggctccggacactttg cttcaggacgagggagaggctacg	55	6, 7, 8	96, 215, 679	(ac)8, (ca)8
IpSTS00006	BV078117	*amyloid beta (A4)-like protein 2*	actctgattggcttgctggt attctgtggccccatatcac	52	1	459	(at)30, (ac)13, (ac)23, (gt)35
IpSTS00007	BV078118	*Ribosomal protein S16*	caggtgttgatatccgattgcgtg tccgaacttcttggactcgcag	56.4	3, 4	229, 842	(gt)8
IpSTS00008	BV078111	*Myosin light chain 2*	ggcaaagaagaaggaagctg catgctgaggaagatggtga	55	1, 2	125, 111	(ac)12
IpSTS00009	BV078119	*B-cell receptor-associated protein 37*	agggccatcatcttcaacag tggtagaggaagggcaggtt	55	1, 2	243, 31	None
IpSTS00010	BV078120	*Translation initiation factor 2 eTIF2*	gaagaaggcgatggagtcag gcaacatcaggtccaagtca	56	1, 2	394, 237	None
IpSTS00011	BV078121	*Lymphocyte cytosolic protein 1 (L-plastin)*	agtagtagcttgccggtgct actcgagctccgatcttcct	55	1	330	None
IpSTS00012	BV078122	*Methyltransferase*	tggttccagagacacattcg attacccgtgtgaggcaaac	55	1, 2	27, 85	None
IpSTS00013	BV078123	*MCT-1 oncagene*	gtggagcgaatatcatgtgc gccacagtccatcattgaga	55	2, 3	134, 105	None
IpSTS00014	BV078124	*Cellular retinoneic acid-binding protein*	tcagctgtgagcaaacccta cgtcatctcctcaacttgcat	53.2	1	452	None
IpSTS00015	BV078125	*Myosin light chain 1*	acaggagatgggaggatctg attactgtgccgttgccttc	56	1	149	None
IpSTS00016	BV078126	*CC chemokine-1*	cgggaaagctgagaaacaac tgcatacacatccaagtcac	52.4	1, 2, 3, 4	174, 104, 119, 209	None
IpSTS00017	BV078127	*Ubiquitin specific protease 9*	acgtgcagttcatgcacagc actgtgtgcaaaccagcagc	56.4	1	1028	None
IpSTS00018	BV078128	*Ribosomal protein L3*	cttccccaaagatgacccaacc tcatctcgtcctggctgaacac	61	1	1078	None
IpSTS00019	BV078129	*Human cDNA DKFZp434K098*	ctgaccaacttcagctgcaacg tgatgcaattatccgccaaccac	55.5	1	1069	None
IpSTS00020	BV078130	*Ribosomal protein S11*	aggtcttatcagaagcagcccac tcaaaacggttgtacttgcggatg	56.4	1, 2	222, 287	None
IpSTS00021	BV078131	*Trypsin IA*	gttggctatcacttctgcggtg agggtgagagttattgcagtctgtg	57.5	1, 2	86, 215	None
IpSTS00022	BV078132	*Ribosomal protein S30*	ctctagagggtctttgtgtggagg cttctttccaaaggtgggcacc	57.5	2	323	None
IpSTS00023	BV078133	*Ubiquitin-conjugating enzyme 7*	cgaatatcctgacatggcaaggctc tctctgcactaattacaggcagacag	54.9	1	183	None
IpSTS00024	BV078134	*Ribosomal protein S2*	tgctctaaagaggtggccacag ctggtgtgagttttggccagatg	58.1	3, 4	102, 400	None
IpSTS00025	BV078135	*Adenine nucleotide translocase*	cgccatttctttcgccaaggac agatgccgaagtagctgctctg	57.5	1	1272	None
IpSTS00026	BV078136	*Elongation factor 1 alpha*	cgactctggaaagtcaaccaccac tgatctcctcaaagcgagcctg	58.1	1	838	None
IpSTS00027	BV078137	*Troponin T*	tcagaacaaggatctcgtggaactg cttctgcaggtagctgctgaac	57.1	2, 3	120, 91	None

Table 1 Continued.

STS no.	Accession no.	Gene identity	PCR primers	Annealing temp °C	Introns	Size of intron (bp)	Microsatellite
IpSTS00028	BV078138	*Pseudouridine synthase 1*	actatcgaagatgaactggtcactgc agagaacgcgatagtgggaagc	55.5	2, 3	168, 183	None
IpSTS00029	BV078139	*Ribosomal protein P0*	agatgcagaccatccgtctgtc tggtgatacccaaagcctggaag	56.4	2	1066	None
IpSTS00030	BV078140	*Ferritin heavy subunit*	ggtgagacagaatttccaccaggac tctccagctgtagtgcacactc	57.1	1, 2	300, 388	None
IpSTS00031	BV078141	*Breast adeno-carcinoma marker*	tggagtttgagcgccaaagtg agcatctgcatctgccaaagc	54.9	3, 4	154, 97	None
IpSTS00032	BV078142	*Ubiquitin-conjugating enzyme 9*	gctgttccaactaagaaccctgatgg gtcctcctctaaaatggagaggcac	55.9	2	558	None
IpSTS00033	BV078143	*Ribosomal protein S3*	gcatcttcaaagccgagctgaac gtgtctacgtaatagttgacggggtc	62	2, 3, 4	157, 320, 465	None
IpSTS00034	BV078144	*Pyruvate dehydrogenase*	acgtactacatgtcagccggtc cgcagacaattcaaaggccatgc	57.5	4	1354	None
IpSTS00035	BV078145	*Heat shock protein 70*	agtctgagaatgttcaggacctgc tccgccttgtatttctcagcctc	61	2, 3	115, 83	None
IpSTS00036	BV078146	*Ribosomal protein L10a*	agtatctggaaactgtggagctgc ggtattcaggttctcgttgtgggtg	56.4	1, 2	496, 600	None
IpSTS00037	BV078147	*Interleukin-8*	cacgatgaaggctgcaactc cattgtgtaacgaactgtgtg	55	1, 2, 3	99, 164, 113	None
IpSTS00038	BV078148	*Methionine-tRNA synthetase*	agccgactcattaacgctgt tggcggttatggagaggtag	56.7	2	175	None
IpSTS00039	BV078149	*Translation initiation factor 3 subunit 9*	gagcattacatggcctctg ggactacagatctccgagtg	56	4, 5, 6, 7	116, 107, 89, 513	None
IpSTS00040	BV078150	*S-phase kinase-associated protein 1A*	gggacgttgaaatcgccaaacag tggaaaactcctgatcccacacag	55.4	1, 2	172, 1021	None
IpSTS00041	BV078151	*Ribosomal protein L9*	gttcaaccacatcaacctggagc tgatctccactagagagccagtctc	56.4	2, 3	148, 116	None
IpSTS00042	BV078152	*Processing peptidase beta subunit*	cagctcaagtgtcgctcaac cttcctaaagcggtgtcctg	53	1, 2, 3	486, 114, 314	None

Table 2 Analysis of polymorphisms of the gene-associated microsatellites.

Locus	PCR primers[1]	No. fish tested	No. alleles	Heterozygosity[2]
IpSTS00001	*Tail*-AATGAGATCAAGGACAGCGTG *GTTT*-GGCATCTGTGTGAAGTGTAAC	33	21	0.925
IpSTS00002	*Tail*-GGTCACACTCTACGGCACAC *GTTT*-ATTACCGCTAGTTTGAAGAGTG	36	12	0.890
IpSTS00003	*Tail*-GATTATAGAGGCTGATGTGCGAG *GTTT*-CATGTTGGGTCAGGCGGAAG	37	3	0.538
IpSTS00004	*Tail*-CGGCAAATTGAGAAGCTGCG *GTTT*-CAGCAGCTGTGGGGATGTG	33	15	0.905
IpSTS00005	*Tail*-AGCGACGCATCCTCACTGAG *GTTT*-AGCTGAGAGAGGTGAGAGTG	37	12	0.870
IpSTS00007	*Tail*-GCAGTATCAGTACTCACACTTC *GTTT*-CCTCAGAGTACTCCATCAAC	35	6	0.654
IpSTS00008	*Tail*-CTTCACCATCATGGACCAG *GTTT*-GAAGTTTATTGGACCTGGAGC	36	7	0.764

[1]*Tail* indicates the sequence 'GAGTTTTCCCAGTCACGAC' used to facilitate fluorescent labelling of PCR products. *GTTT* was added to promote non-template adenylation by *Taq* polymerase.

[2]Heterozygosities were determined in outbred catfish from commercial populations.

Table 3 Linkage analysis of the gene-associated microsatellites. Genotyping was conducted in two full-sib families, family 1 and family 2, as previously described[3].

Locus	Informative meioses	Linkage group	Closest marker	Recombinant fraction	LOD
IpSTS00001	70	U21	*IpCG0148*	0	20.77
IpSTS00002	72	U12	*IpCG0222*	0.17	19.68
IpSTS00003	142	U4	*IpCG0002*	0.07	13.22
IpSTS00004	272	U20	*IpCG0099*	0.09	11.62
IpSTS00005	209	U6	*IpCG0079*	0.05	28.22
IpSTS00007	260	U19	*IGF 1*	0.23	8.00
IpSTS00008[1]	0	–	–	–	–

[1] *IpSTS00008* was not polymorphic in either family.

(Table 3) while *IpSTS00008* was not polymorphic in either family.

Comments: Sequencing 42 introns produced eight microsatellites that are associated with the selected genes. Thus, intron sequencing can be used to develop type I markers in catfish with a success rate of about 19%. While the efficiency was low, this approach may be useful for targeted microsatellite development within known genes. Because a large number of ESTs are available from catfish,[4] large numbers of gene-derived type I markers could be developed with the assistance of intron-prediction software.

Acknowledgements: This project was supported by a grant from USDA NRI Animal Genome Basic Genome Reagents and Tools Program (USDA/NRICGP 2003-35205-12827). Jerry Serapion was partially supported by a specific cooperative agreement from USDA, ARS, Catfish Genetics Research Unit.

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doi:10.1111/j.1365-2052.2004.01189.x

Exclusion of *WT1* as a candidate gene for canine *SRY*-negative XX sex reversal

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Accepted for publication 13 August 2004

Source/description: In mammals, the Y-linked *SRY* gene is normally the genetic signal for testis induction. An abnormality in sex determination presents an exception to this rule. In *SRY*-negative XX sex reversal, which represents approximately 20% of XX sex reversal cases in humans, and the majority of cases in dogs and other domestic animal species, the *SRY* gene is not present.[1,2] This suggests that autosomal genes have a role in testis induction.

Intensive research has resulted in the discovery of a handful of genes having roles in the formation of the bipotential gonad and/or subsequent gonadal differentiation.[3] The Wilms tumour gene (*WT1*) encodes a zinc-finger, DNA binding protein that plays a critical role in kidney and gonadal development.[4,5] Three disorders in humans (WAGR syndrome, Denys–Drash syndrome and Frasier syndrome) are associated with *WT1* mutations.[6] Kidney failure and gonadal dysgenesis associated with male to female sex reversal (46,XY female) are striking features of these syndromes. Recent evidence suggests that during gonadal development *WT1* upregulates *SRY* transcription by directly binding to DNA.[4]

A tetranucleotide repeat (GAAA) of approximately 12 bp was previously identified in canine *WT1* (GenBank accession no. U00687)[7] in a region homologous to human intron 7 (http://www.ensembl.org). The objective of this study was to determine whether alleles for this microsatellite marker within canine *WT1* are associated with the affected phenotype in a pedigree of canine *SRY*-negative XX sex reversal.

Primer sequences:
Forward: 5′- GGT TTG TAG GCT CGC TTC TCA TTG -3′
Reverse: 5′- CTA GCG GGA TCC CAC TGT TCT GT- 3′

The forward and reverse primers were designed with Primer Select software (DNASTAR, Madison, WI, USA). The forward primer was 5′-end labelled with a HEX fluorescent tag (IDT, Coralville, IA, USA).

PCR conditions: Amplification was performed using 50 ng of canine genomic DNA template, 1 μm of each primer, 0.25 mm dNTPs, 3 μl of 10 X PCR buffer (Perkin-Elmer Life Sciences, Foster City, CA, USA), 3.0 mm $MgCl_2$ and 1.5 U Taq polymerase (Ampli Taq II, Perkin-Elmer Life Sciences) in a reaction volume of 30 μl. After initial denaturation at 95 °C for 5 min, the samples were amplified for 35 cycles at 95 °C for 30 s, 70 °C for 1 min, 72 °C for 1 min with a final extension at 72 °C for 2 min.

A portion (1.5 μl) of each PCR product was pooled with 13 μl formamide and 0.5 μl of the TAMRA internal size standard (PRISM Genescan-500; Applied Biosystems, Warrington, UK). Denatured samples were analysed on the ABI310 Genetic Analyzer using filter set C (Applied Biosystems, Foster City, CA, USA) and product sizes relative to the internal